

Supplementary material

High RIG-I expression in ovarian cancer associates with an immune-escape signature and poor clinical outcome

Dominik Wolf, Heidi Fiegl, Alain G. Zeimet, Verena Wieser, Christian Marth, Susanne Sprung, Sieghart Sopper, Gunther Hartmann, Daniel Reimer, Maximilian Boesch

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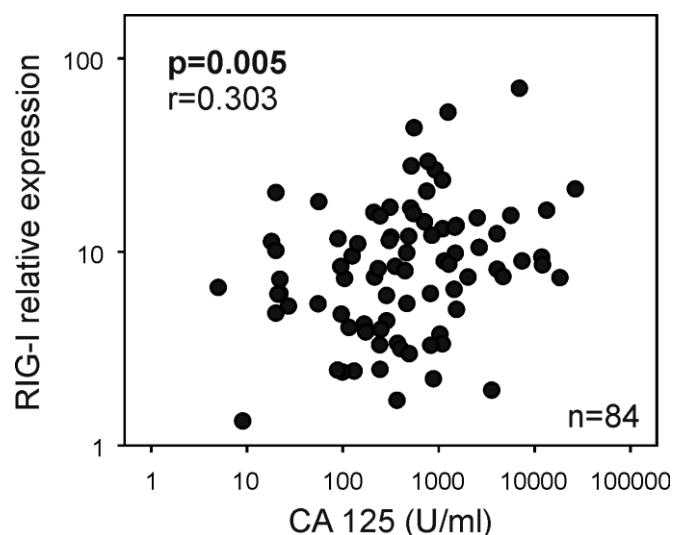
Supplementary tables

Supplementary table 1. Primers and probes used for quantitative real-time PCR.

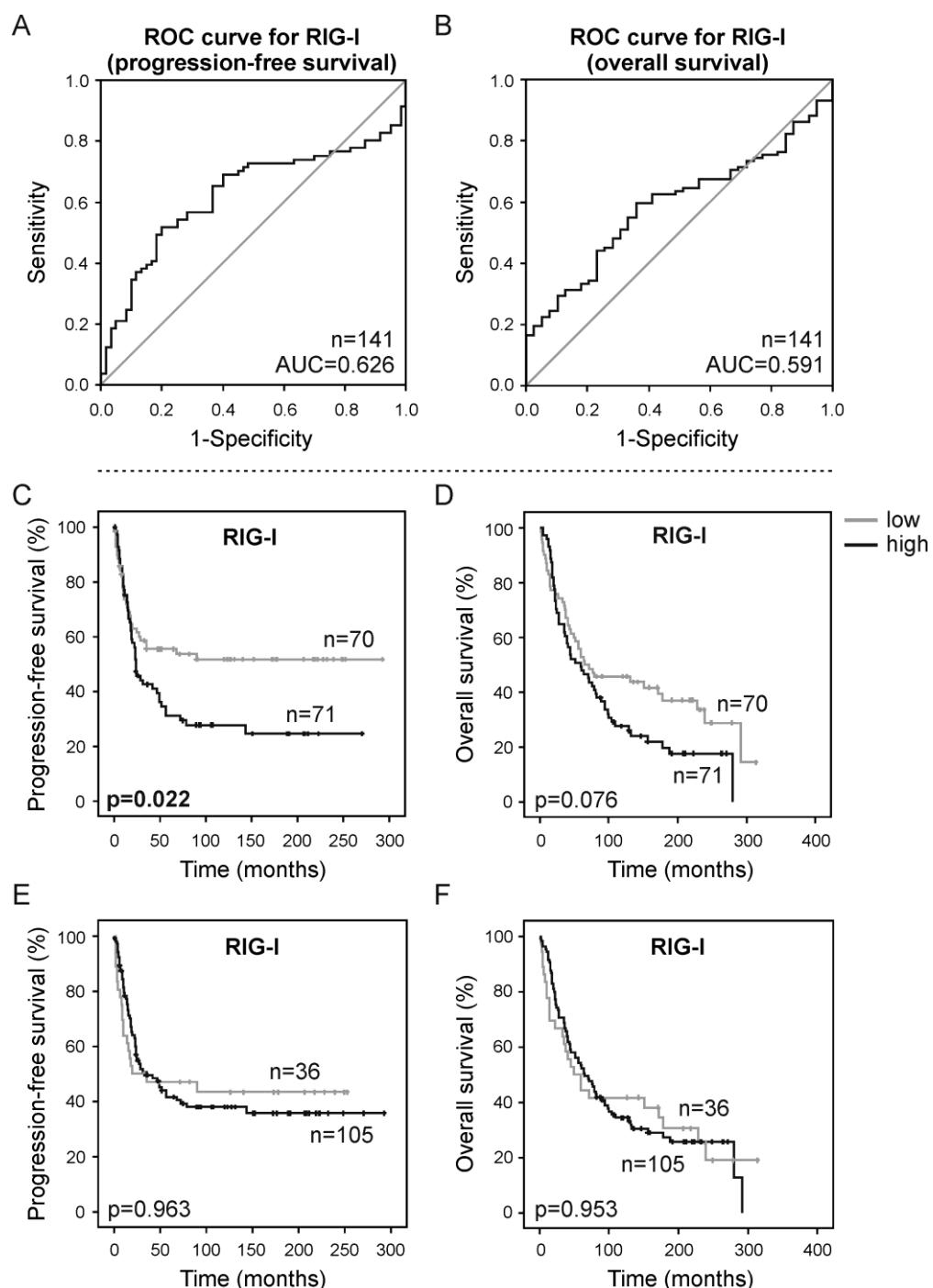
Gene (alias)	Oligonucleotide	Sequence/assay ID	Manufacturer
ADAR (ADAR1)	—	Hs00241666_m1	Thermo Fisher Scientific
ADARB1 (ADAR2)	—	Hs00953724_m1	Thermo Fisher Scientific
CD274 (PD-L1)	Sense	5'-AATGATG GATGTAAAAATGTGG-3'	Metabion International AG
	Antisense	5'-AATGCTGGATTACGTCTCCTCC-3'	
	Probe	5'-FAM-TCCAAGATACAAACTCAAAGAAGCAAAGTGATACACATT-TAMRA-3'	
DDX58 (RIG-I)	—	Hs00204833	Thermo Fisher Scientific
EZH2	—	Hs00544833_m1	Thermo Fisher Scientific
FOXP3 (FoxP3)	Sense	5'-TGGCTAGGAAAATGGCA-3'	Metabion International AG
	Antisense	5'-GCAGGAGCCCTTGTGCG-3'	
	Probe	5'-FAM-TGACCAAGGCTTCATCTGTGGCATCA-TAMRA-3'	
IFNA1 (IFN-α1)	—	Hs03044218_g1	Thermo Fisher Scientific
IFNA2 (IFN- α2)	—	Hs00265051_s1	Thermo Fisher Scientific
IFNB1 (IFN-β)	—	Hs00277188_s1	Thermo Fisher Scientific
IFNG (IFN-γ)	—	Hs00174143_m1	Thermo Fisher Scientific
IRF1	Sense	5'-TTTGTATCGGCCTGTGAATG-3'	Metabion International AG
	Antisense	5'-AAGCATGGCTGGACATCA-3'	
	Probe	5'-FAM-CAGCTCCGGAACAAACAGGCATCCTT-TAMRA-3'	
IRF2	Sense	5'-CGCCCCTCGGCACTCT-3'	Metabion International AG
	Antisense	5'-TCTTCCTATGCAGAAAGCGAAC-3'	
	Probe	5'-FAM-TTCATCGCTGGCACACTATCAGT-TAMRA-3'	
PDCD1 (PD-1, CD279)	—	Hs01550088_m1	Thermo Fisher Scientific
TBP	Sense	5'-CACGAACCACGGCACTGATT-3'	Metabion International AG
	Antisense	5'-TTTTCTTGCTGCCAGTCTGGAC-3'	
	Probe	5'-FAM-TGTGCACAGGAGCCAAGAGTGAAGA-BHQ-3'	

Supplementary figures

Supplementary figure 1. RIG-I expression correlates with ovarian tumor load. RIG-I levels were correlated to the tumor marker CA 125, detected on protein level in serum at the time of diagnosis. Abbreviations used: RIG-I, retinoic acid-inducible gene-I.



Supplementary figure 2. Defining an optimal cut-off for survival analysis. (A+B) ROC curve analysis of RIG-I expression for PFS and OS. (C+D) RIG-I expression was dichotomized according to 50th percentile statistics and PFS and OS was analyzed for the whole cohort using the Kaplan-Meier methodology (n=141). (E+F) RIG-I expression was dichotomized according to 25th percentile statistics and PFS and OS was analyzed for the whole cohort (n=141). Abbreviations used: AUC, area under the curve; PFS, progression-free survival; OS, overall survival; RIG-I, retinoic acid-inducible gene-I; ROC, receiver operating characteristic.



Supplementary figure 3. Validating the prognostic significance of RIG-I expression.
 (A+B) Kaplan-Meier analysis of PFS (n=614) and OS (n=655) in an independent cohort of OC patients using TCGA data available at www.kmplot.com/ovar. RIG-I mRNA expression was detected using a probe with the annotation 222793_at. The algorithm selected the best cut-off for dichotomization (function ‘Auto select best cutoff’), which happened to be the 73rd percentile for both PFS and OS. Abbreviations used: OC, ovarian cancer; OS, overall survival; PFS, progression-free survival; RIG-I, retinoic acid-inducible gene-I.

